**Cohort Profile: Siyakhula Cohort, rural South Africa**

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**Summary**

Each year millions of children in low and middle-income countries (LMIC) fail to reach their developmental potential due to factors including poverty, malnutrition, poor stimulation and HIV. Although vertically-acquired HIV can now be prevented, little is known about the impact of HIV exposure in fetal and early life on the development of the many HIV-negative children. The Siyakhula Cohort was established within the Canadian Grand Challenges ‘Saving Brains’ initiative, to support re-enrolment of strategic cohorts in LMIC. This unique cohort in rural South Africa includes 1,536 HIV-negative children born to HIV-infected (HIV-exposed) and HIV-uninfected( unexposed) mothers, enrolled from the Africa Health Research Institute (Africa Centre) – formerly the Africa Centre for Population Health.. The cohort includes data on HIV-exposure in fetal and early life, and other early life factors (including breastfeeding) known to impact on later health outcomes. At birth, all children benefited from the early Prevention of Mother-to-Child-Transmission of HIV services in the district, while a subgroup were part of an additional early life breastfeeding intervention, the Vertical Transmission Study (VTS). This cohort predated antiretroviral treatment (ART) roll-out, allowing for examination of outcomes associated with HIV exposure, without ART exposure in utero and during breastfeeding. Current assessments at ages 7-11 years collected data on growth, health, cognition (including executive function), education, and emotional-behavioural outcomes at primary school-age.

**Why was the study set up?**

The Africa Centre, one of 11 sites from low-middle income countries (LMIC) to receive funding from Grand Challenges Canada, established the Siyakhula cohort to examine associations between early life factors and later child development, specifically exposure to a breastfeeding intervention[1](#_ENREF_1), [2](#_ENREF_2) and later child development, allowing for HIV-exposure in fetal and early life.

Evidence linking breastfeeding with improved cognition is conflicting, with EBF linked with improved cognition in a meta-analysis[3](#_ENREF_3), but inconsistent results in a systematic review depending on study design and methodology[4](#_ENREF_4). There is also a dearth of evidence on the effect of HIV on HIV-exposed but HIV-uninfected children. A recent systematic review[5](#_ENREF_5) examining HIV-exposure and child development found data from only 11 studies worldwide (1,591 children aged 0-18 years; 650 HIV-exposed; 736 HIV-unexposed; 205 HIV-infected). The review concludes that HIV-exposed children are disadvantaged in terms of child development, in particular emotional-behavioural development, compared to their HIV-negative unexposed peers. However, findings were inconsistent, with most evidence based on small samples with wide heterogeneity in outcome measures. There are few longitudinal studies, almost none with HIV-negative controls or a population norm, and no studies on primary school-aged children.

The Siyakhula cohort was established in 2012 from the Africa Centre ([www.africacentre.ac.za](http://www.africacentre.ac.za)) research platform, in a rural, high HIV prevalence setting[6](#_ENREF_6). With the scale-up of HIV treatment programmes, parents are surviving to care for their children, and mother-to-child transmission (MTCT) of HIV has been virtually eliminated[7-10](#_ENREF_7). Children in the Siyakhula cohort were born in the pre-ART era, between 2001 and 2005, in the Hlabisa sub-district, and are all HIV-negative. Some children had previously participated in the VTS, which supported mothers with exclusive breastfeeding (EBF) for the first six months of life, and demonstrated that EBF reduced the risk of MTCT of HIV compared to mixed breastfeeding[2](#_ENREF_2). Similar-aged children were also enrolled from the Africa Centre Demographic Surveillance System (DSS). These children had been exposed to the same standard of care, including similar messages regarding HIV and early infant feeding according to national guidelines at the time[11](#_ENREF_11), [12](#_ENREF_12), without the additional VTS breastfeeding support. The Siyakhula cohort is well-placed to address the question of whether, in the context of HIV, EBF contributes to improvements in the development and health of children.

**Who is in the cohort?**

All children in the cohort are HIV-negative. We excluded HIV-positive children, as they have HIV-specific and unique developmental risks[5](#_ENREF_5), [13](#_ENREF_13). Children were eligible for enrolment in the Siyakhula cohort if HIV-negative, 7-11 years of age, born and still resided in the study area (the Hlabisa sub-district), if their mother’s HIV status during pregnancy was known, if mothers received antenatal care for the index child in the study area, and both mother and child were still alive.

The children included in the Siyakhula cohort came from two different sources. Firstly we enrolled HIV-exposed and unexposed children who met the above eligibility criteria from the VTS. The children in the VTS had their final study visit when they were two years of age. At the end of the VTS in 2006, 1289 children were still alive, were known to have mothers who were alive, and were themselves HIV-negative (see Figure 1). The VTS enrolled children from the Hlabisa sub-district (see Figure 2).

The second source of children for the Siyakhula cohort came from the Africa Centre Demographic Surveillance Area (DSA), situated in part of the Hlabisa sub-district (see Figure 2)[6](#_ENREF_6). Since 2000, the Africa Centre has collected data bi-annually (tri-anually since 2012) from almost 90,000 people in 11,000 households per round. In 2003, an annual HIV surveillance was added, with HIV status collected on consenting adults[14](#_ENREF_14). The children from the DSA had been born between 2001-2005 as had those in the VTS, but had not taken part in the VTS (so had not received the EBF intervention). Within the DSA 1226 children were documented at their last surveillance visit to be alive, with mothers who were alive, and were HIV-uninfected (see Figure 1).

It is important to note that all eligible children within the DSA were approached for inclusion in the Siyakhula cohort – some of whom had participated in the VTS and others who had not. However in addition, the Siyakhula cohort included some VTS children who lived in the Hlabisa sub-district but in areas outwith the DSA (see Figure 2).

Therefore, four groups of HIV-negative children were recruited: HIV-exposed and unexposed from the VTS, and HIV-exposed and unexposed from the DSA .The consort diagram (Figure 1) shows the pool of 2,515 potential participants, those who enrolled (n=1,592), and those who completed assessments (n=1,536). Table 1 shows the characteristics of those who were enrolled compared to those who were not enrolled. Of the 1,536 children who completed all assessments, 1059 were HIV-unexposed and 477 HIV-exposed at birth.

***Insert Figure 1, Figure 2 and Table 1 here***

**How often have they been followed up?**

Data for Siyakhula have been collected over three visits between September 2012 and June 2014, when the child was between seven and 11 years of age. Study consent was obtained in Visit 1, socio-economic and health data, mothers’ mental health and cognitive ability in Visit 2, and children’s cognition and executive function assessed in Visit 3. When the mother was not the primary caregiver, mental health assessments were completed by the child’s primary caregiver during Visit 2. Differences between those lost to follow-up and those who completed assessments are shown in Table 1. Children who are part of the DSS also have longitudinal data available, collected biannually.

**What has been measured?**

Table 2 describes the measures used and data collected.

***Insert Table 2 here***

Child cognition was measured using the Kaufman Assessment Battery for Children 2nd Edition (KABC-II), a validated measure of cognitive development in children aged 3-18 years (Table 3)[15](#_ENREF_15). The test battery was implemented using the Luria model theoretical approach, well-suited to children in low-income, cross-cultural, settings where quality and exposure to school may vary. Eleven sub-tests were administered including both verbal and nonverbal tests for all domains. Sub-tests were scored into four index scales, covering all aspects of cognition and used to calculate a mental processing index (MPI) reflective of general intelligence.

The KABC-II test battery is licensed to Pearson Ltd. USA[15](#_ENREF_15), and test kits and forms were purchased. All subtests in the Luria Model battery were retained, without adaptation, while the administration manual was translated, under license from Pearson Ltd, with fees waivered[16](#_ENREF_16). An expert review team, including the authors of the KABC-II, selected the sub-tests considered most culturally appropriate, and one sub-test substitution was made (Atlantis and Atlantis delayed tests replaced Rebus/Rebus delayed tests). One additional sub-test from the knowledge scale was included as a supplementary test of vocabulary and general knowledge.

Three additional subtests to the KABC-II were added to test executive function capacities: working memory, inhibition and switching (Table 3). These subtests were drawn from the Neuropsychological Assessment Battery 2nd Edition (NEPSY-II)[17](#_ENREF_17) also licensed to Pearson Ltd. USA[18](#_ENREF_18). Selective assessments used individual sub-tests in the NEPSY battery (Attention and Executive Function Domain) considered appropriate for focused evaluation of neuropsychological functioning. Test kits and forms were purchased; tests were used in their original format, while auditory stimuli were translated under translation license from Pearson’s, again with fees waivered[18](#_ENREF_18).

***Insert Table 3 here***

Children’s emotional and behavioural problems were measured using the Parent Report versions of the Child Behaviour Checklist (CBCL) for children aged 6-12 years, which has been validated in over 30 countries including South Africa[19](#_ENREF_19), [20](#_ENREF_20). The CBCL, licensed to the Achenbach System of Empirically Based Assessment (ASEBA), offers a comprehensive approach to assessing adaptive and maladaptive functioning and was used with permission and translation licence.

The CBCL comprises two parts: Part 1 includes a competencies questionnaire, including questions on children’s engagement in academics, sports, hobbies, and the quality of their friendships and sibling relationships. CBCL Part 1 is time intensive and seldom used in a research context, but mostly provides data for clinical interpretation and treatment. We used an abbreviated version, collecting qualitative data on children’s social and peer competencies which were coded and categorized to be used in quantitative analysis.

The CBCL Part 2 behavioural problems rating scale was implemented in full, including a 120-item rating scale which make up a composite Total problems score; a high score indicated more problems. The parent rated the child behaviour on a three-point scale on a series of symptoms which represent eight psychological syndromes. There are 113 numbered items, but item 56 has 7 sub-items on somatic symptoms, making 120 items in total. The items are scored as: 0= not true (as far as you know); 1=somewhat or sometimes true; 2= very true or often true. Some items, if endorsed, include qualitative descriptive answers on the child’s problem behaviour. These descriptions are not used in the scoring system and are only of clinical interpretative value only.

In Siyakhula, CBCL scores were normed using multicultural Rating-to-Score norming software (purchased from ASEBA) to produce normed t-scores for the Total score, and the two subscales, and for the six Diagnostic and Statistical Manual (DSM) disorders, such as Internalising problems including Affective, Anxiety and Somatic disorders and Externalising problems includingAttention Hyperactivity (ADHD), Oppositional and Conduct disorders. Cronbach’s reliability was high (α = 0.94) exceeding α = 0.75 recommendation for standalone measure.

Research assistants with 5-7 years of research experience, administered the assessments following two weeks’ training. Quality assurance and reliability checks were conducted by two Master’s level psychology graduates. Rater reliability was assessed against a gold standard assessor for a subsample of 10% of assessments, with reliabilities ≥80% for all assessors throughout the data collection period.

**What has it found?**

Analysing the VTS children only, we reported that longer duration of EBF (6 vs. <1 month) was associated with fewer than average conduct disorders, and weakly associated with improved cognitive development in boys[21](#_ENREF_21). In addition, HIV-exposed children performed as well as HIV-unexposed children in the domains examined (cognition and emotional/behavioural development). Maternal intelligence quotient (IQ) was strongly associated with children’s later cognitive development, an interesting finding as maternal IQ is seldom included as a confounding variable in long-term breastfeeding studies, particularly in LMIC[4](#_ENREF_4).

This is, to our knowledge, the largest cohort of HIV-exposed and unexposed children in Africa who have completed a full battery of cognitive and executive function tests[5](#_ENREF_5), [22](#_ENREF_22), [23](#_ENREF_23). The sample size is similar to most normative samples in high-income countries[15](#_ENREF_15). We considered it necessary to use a full battery of tests given the absence of normative or reference developmental data in African populations. The approach provides an opportunity for children to perform on at least two sub-tests in each domain of intelligence, including both verbal and non-verbal tests, substantially reducing the risk that performance is a consequence of test specific variables, or due to cultural or school exposure.

 We used structured equation modelling (SEM) techniques to test the psychometric validity of the child cognitive measures. This is an important step when using child development batteries in new populations where they have not been tested before. Such techniques also help us to understand the underlying constructs measured by a number of different subtests. The battery has a pre-determined set of sub-scales and 10 sub-tests which are based on well-established theories (Luria) of cognitive development. We therefore used confirmatory factor analysis (CFA) to load each specific sub-test onto one of four latent factors. Each latent factor represents a domain of cognition. Thus, which latent factor a sub-test was loaded onto depended on which of the four domains of cognition the sub-test is designed to measure (see Figure 3). SEM is a useful technique to test whether the data in the cohort fits with the expected theoretical model of cognition embedded within the KABC design, i.e. that specific sub-tests measure one of four key cognitive skills. In addition, SEM techniques separate the construct-related variance from sub-test task demands (for example, ability to count or use a pen and paper). This is because the latent variables represent the shared variance across different tasks which measure the same construct in different ways.

We also examined the three NEPSY subtests using CFA and found them to represent individual factors. The NEPSY scales are designed to measure executive function, and, in order to improve the KABC factor on planning we included the NEPSY subscales along with the KABC subscales, combining the NEPSY executive function tests together with the Planning scale of the KABC (Figure 3). The planning scale reflects a measure of executive functions, hence the factor structure of the test battery reflects a strong battery of both cognition and executive function. The fit of this final model was also acceptable (Comparative fit index[24](#_ENREF_24) (CFI) 0.947, Root-mean square error of approximations[25](#_ENREF_25) (RMSEA) 0.047, [0.042, 0.051]). Goodness of fit was determined in accordance with Hu *et al* and was indicated by CFI and Tucker-Lewis fit index[26](#_ENREF_26) (TLI) values of over 0.95, and RMSEA of less than 0.06. Multiple indices were used as they provide a more comprehensive evaluation of model fit. The factors were all highly correlated with each other demonstrating the inter-relatedness of these cognitive capacities and the importance for latent factor modelling to separate out any specific effects.

***Insert Figure 3 here***

In examining children’s performance, Figure 4 shows the mean scores for the KABC sub-tests by age, comparing expected versus observed scores from the Siyakhula Cohort. Children’s expected scores are derived from the KABC-II normative tables, which indicate the expected score for age at a sub-test level.

***Insert Figure 4 here***

Overall the developmental scores of the cohort were normally distributed, with the upper tail of the cohort performing in a similar range to the average-to-average children’s scores in HIC. However, the vast majority of children in Siyakhula performed substantially lower than their age equivalent US counterparts. While some of this could be accounted for by variations in school exposure and quality, the differences are nonetheless substantial, and widen with age, suggesting that these children would face particular disadvantages within educational settings.

Since children performed poorly across most sub-tests, one would not expect this poor performance at a scale level to be accounted for by sub-test or stimuli effects. One exception is children’s improved performance on the number recall test, in the Sequential scale, which tests a child’s capacity to retain and store information, and use it again within a few seconds. The scale has three sub-tests that use non-verbal hand movements, numeric and verbal stimuli. Children performed well on number recall but consistently poorly on the other two subtests (Figure 4). This likely reflects a higher exposure to number recall tests, common even in poor quality primary schools. Number recall does not reflect numeracy skills, instead these are better reflected by performance on the Simultaneous scale, where children’s performance was again consistently poor across all subtests, although this difference was less marked on the block counting tests, perhaps reflecting that rural children were more familiar with the skills required for this assessment.

The cognitive performance in Siyakhula raises significant concerns for the developmental potential of children in these high risk populations. Children’s performance on the Learning scale, which reflects how well children learn, store and retrieve new information, critical to educational success, shows that children are disadvantaged from school entry age, and that differences remain large across the age groups. On most scales, with increasing age, children become more disadvantaged, and while this reflects the expected cumulative nature of learning, or the absence thereof, it also points to the potential advantages of high quality educational interventions in the early years, to ensure that differences are minimised.

**Future plans for the cohort**

We are seeking funding to follow up the cohort into their adolescence years to examine development and growth, and the effect of EBF on later outcomes. An important area of future research will focus on the development of executive function into adolescence. Executive function is a key area of study across the life course, with poor executive function in childhood predicting early mortality, psychiatric disorders, and unhealthy and risky behaviours[27](#_ENREF_27), [28](#_ENREF_28). Children’s executive function predicts adult outcomes, including employment, low intelligence and low social class origins[29](#_ENREF_29), [30](#_ENREF_30). The latter are extremely difficult to modify with interventions, whereas executive function is modifiable to at least 18 years[31](#_ENREF_31). In Siyakhula, oppositional and conduct disorders emerged as the areas of highest mental health risk and to lesser extent child depression. We showed that children’s mental health problems in the areas of conduct disorders were strongly associated with executive functions[32](#_ENREF_32). These data provide a key backdrop against which to examine the role of executive functions and mental health as pathways to risk in early adolescence.

**What are the main strengths and weaknesses of the study?**

**Strengths**

There are almost no large-scale cohort studies in Africa which have used a cross-culturally relevant battery approach to measure children’s cognition in such detail, and very limited data on children’s development in high HIV prevalent areas. A recent systematic review of the global literature on the effects of HIV exposure on child development found only 11 studies from Asia (1), North America (3), Italy (1), and Africa (6) with adequate quality design and measures of standardised cognitive, behavioural and developmental indices[5](#_ENREF_5). Across these studies, cognitive performance, behaviour and developmental delay were measured with 15 different standardised scales from 650 HIV-affected children, 736 control children and 205 HIV-positive children. Our cohort of HIV-exposed and unexposed children (1,536) includes a larger number of children than all exposed and unexposed children (1,386) in the 11 studies published to date. Furthermore, particular critiques of the existing research are directly addressed by our use of a comprehensive cognitive battery, additional sub-tests in critical areas of cognition such as executive function, examination of emotional and behavioural outcomes, and detailed assessment of almost all known confounding factors, including maternal IQ. In addition we have collected data on children’s growth, body fat and blood pressure, all of which predict later health.

Previous studies examining developmental outcomes were unable to adjust for factors known to influence child development, including socio-economic factors, early infant feeding, HIV exposure, and maternal IQ[4](#_ENREF_4). A further limitation of previous studies examining developmental outcomes and early breastfeeding, was the inability to quantify the days of EBF accurately, relying on long periods of maternal recall which has been shown to be inaccurate [33](#_ENREF_33). We have been able to do this for the children who received the VTS intervention, which applied the most stringent of breastfeeding definitions.

South Africa does have one existing longitudinal panel study, the Birth-To-Twenty cohort of children born in Soweto in the late 1990s, with data collected from pregnancy to adulthood[34](#_ENREF_34). Birth-To-Twenty has contributed enormously to our understanding of human development in South Africa and remains a valuable national resource, but the cohort exists in the societal context within which it took place. Children born in 1990 were not able to benefit from many of the interventions implemented since 1994, such as free health care and the child support grant from birth. In addition, the Birth-To-Twenty cohort is an urban cohort recruited early in the HIV epidemic. The children in the Siyakhula cohort are rural, younger, and were born in an HIV epidemic community. Their age and geographical location within an ongoing surveillance platform offers the potential to examine the effects of national interventions such as child support grants, free access to education, water and sanitation on their health. Our cohort thus provides a powerful comparison group, allowing for the investigation of a different time period, geographic location, and policy influences on outcomes.

Finally, there is increasing interest in the outcomes of HIV-exposed but HIV-uninfected children, particularly with the introduction of more complex PMTCT regimens, including maternal ART, during pregnancy and breastfeeding[35](#_ENREF_35). The children in Siyakhula were born before HIV treatment was widely available in South Africa, although single-dose nevirapine was administered as part of the PMTCT programme. This cohort includes HIV-exposed children and provides an important baseline of breastfed children who were not exposed to ART in pregnancy for future studies on the impact of fetal and early life exposure to ART.

**Weaknesses:**

Data are not available on women’s mental health during pregnancy and their mental health in the early childhood period and cannot be inferred from their current measures of depression, anxiety and parenting stress. We have no data on father’s IQ or education which are likely to influence child outcomes. The relatively long time period between birth and current follow-up may limit our ability to examine moderators or other factors along the pathway between early life and these later outcomes. Finally, although this was a population-based sample with a well-defined sampling frame from the DSS, it was a non-random sample, and excluded HIV-positive children. More boys than girls, and more children with HIV-negative compared to HIV-positive mothers were enrolled. Differences between participants who did or did not complete assessments were limited to children born to older mothers being more likely to complete all assessments than children of younger mothers.

**Can I get hold of the data? Where can I find out more?**

Information can be obtained freely from the Africa Centre for Population Health website for researchers who meet the criteria for access to confidential data ([www.africacentre.ac.za](http://www.africacentre.ac.za)). Those interested should contact Dr Kobus Herbst, Deputy Director, Africa Health Research Institute.

**Cohort in a nutshell**

* The Siyakhula cohort, established in 2012, is an observational cohort investigating associations between early life factors (including exclusive breastfeeding and HIV-exposure) and later child development.
* The cohort includes 1,536 HIV-negative, rural African children aged 7-11 years, including 477 HIV-exposed (born to HIV-positive) and 1059 HIV-unexposed (born to HIV-negative) children.
* The cohort includes a wide range of health and developmental outcomes including cognitive development, executive function, emotional-behavioural development, physical growth and biomarkers, adjusting for a range of current and early life factors including infant feeding, HIV-exposure, socio-economic status, school exposure, maternal IQ and maternal mental health.
* One round of data collection has taken place in the Siyakhula cohort (2012-2014), including three data collection visits per child. Further early life data are available on all children, and for children who reside within a large Demographic Surveillance Area limited additional longitudinal data are available biannually since their birth.
* Information can be freely obtained from the Africa Health Research Institute website for researchers who meet the criteria for access to confidential data ([www.africacentre.ac.za](http://www.africacentre.ac.za)), via Dr Kobus Herbst, Deputy Director, Africa Health Research Institute.

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Table 1: Children enrolled and not enrolled, and those who completed and did not complete assessments in Siyakhula

Table 2: Description of data collected in Siyakhula

Table 3: Test battery used to measure child cognition and executive function

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